

# **Biomarker for Prenatal Ethanol Exposure: Identifying a Vulnerable Population**

**Cynthia F. Bearer, M.D., Ph.D.**

**Associate Professor**

**Departments of Pediatrics, Neurosciences &  
Environmental Health Sciences**

**Case Western Reserve University**





# **Ethanol Is the Leading Known Cause of Mental Retardation**

- **Incidence of FAS 1/3,000 live births**
- **1% of all live births manifest FAS or some prenatal alcohol damage**
- **FAS and FASD estimated to cost \$75 million to \$9.7 billion dollars a year**
- **Mechanisms?**



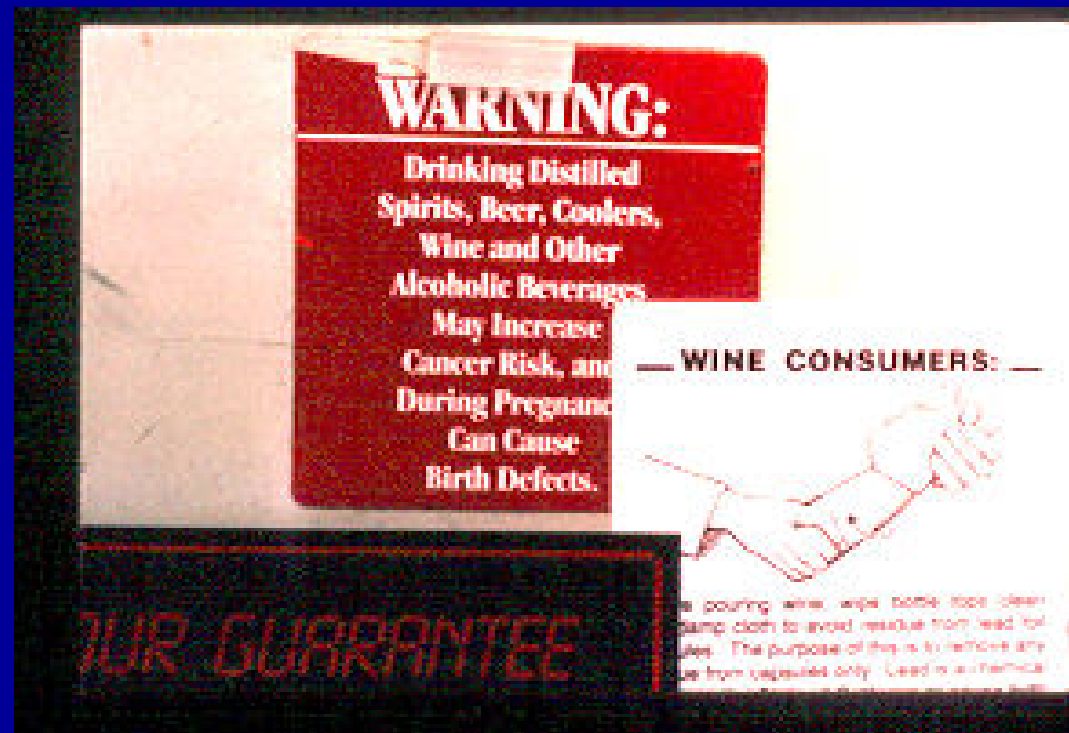
# **Cause: Drinking During Pregnancy**

- **Women most at risk to have alcohol damaged child are those who already have an alcohol damaged child**
- **Role of targeted intervention**
- **Role of early intervention for at risk infants**



# Preventing Drinking During Pregnancy

- Point of sale warnings
- Bottle labels
- Taxation
- Advertisements
- **DON'T IMPACT THE AT RISK POPULATION!**



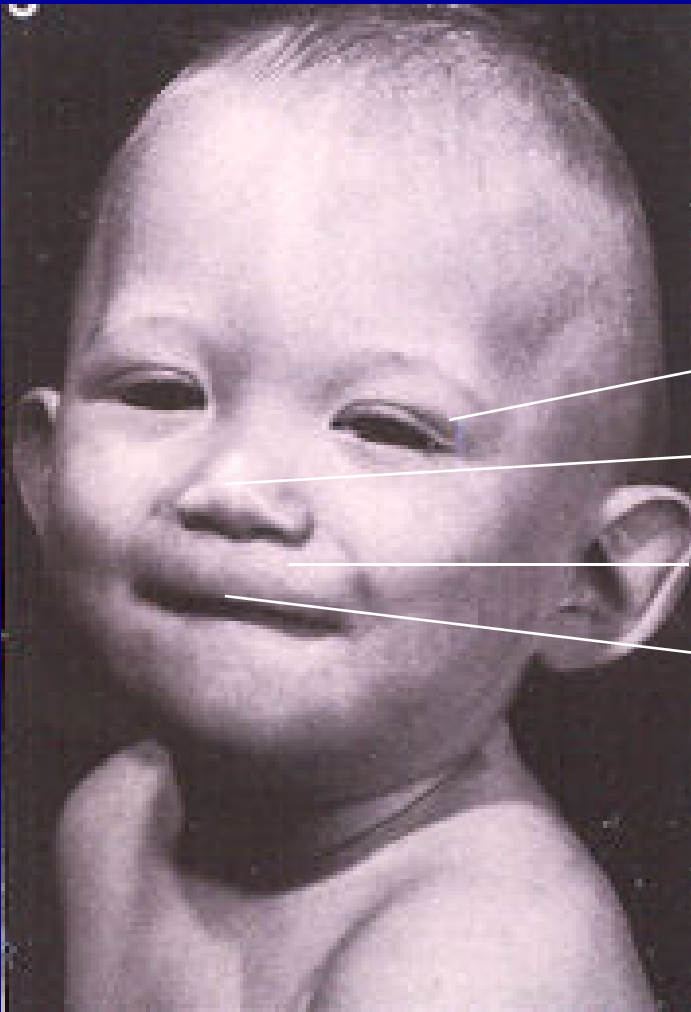


# Why Don't You Just Ask Them If They Drink?

- Underreporting
- Special questionnaires only identify heaviest drinkers (more than 2 drinks a day)
- Social stigmata
- Might be useful in conjunction with other methods



# The Face of Fetal Alcohol Syndrome (FAS)



eyes

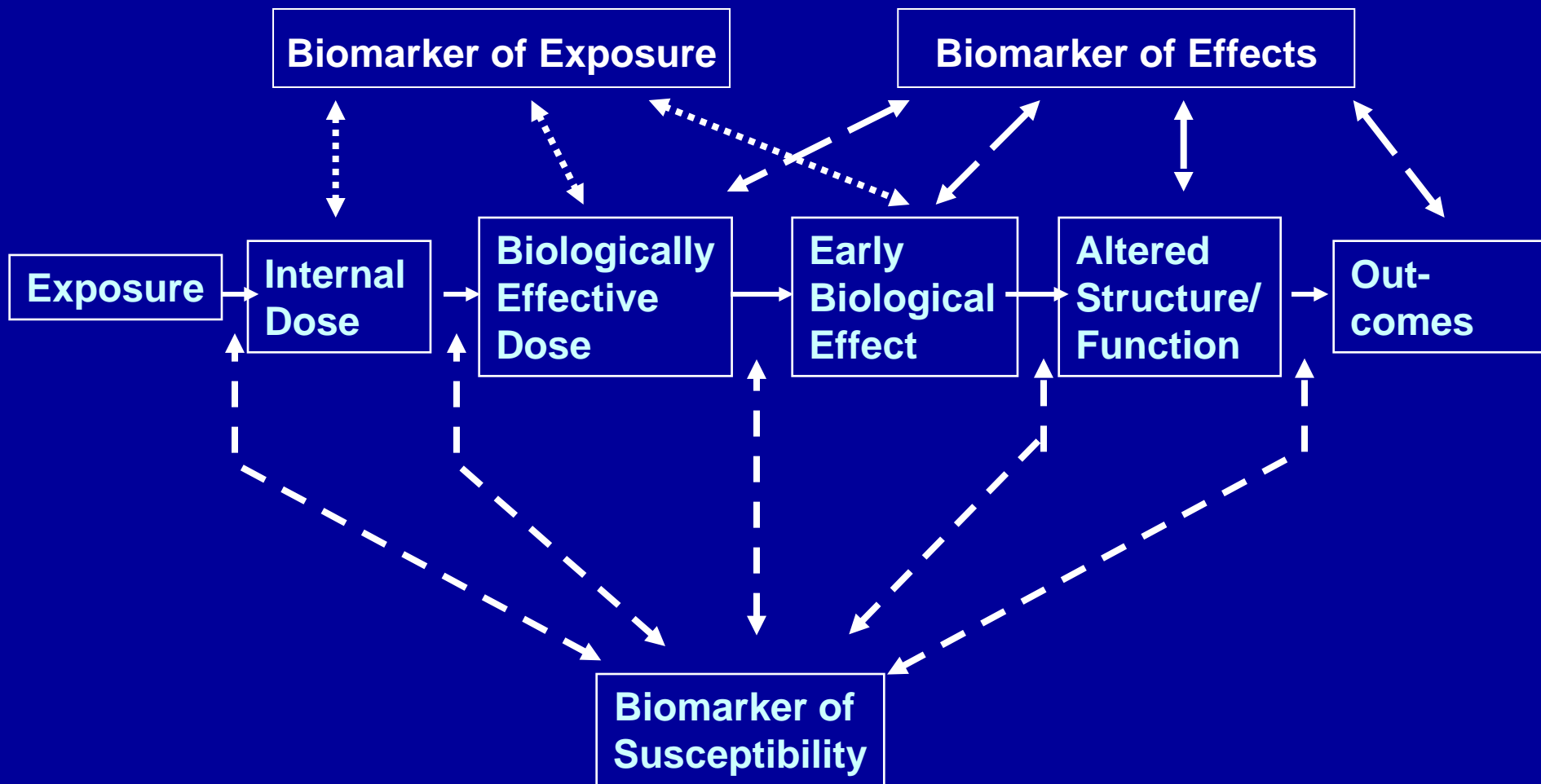
nose

philtrum

lip



# Biomarkers







# Biomarkers

- Indicators of an event in biologic systems or samples
- Three categories
  - Exposure (Blood lead level)
  - Effect (Liver function tests)
  - Susceptibility (Genes)



# Biomarkers

- Need to identify a biological sample (matrix) in which to measure the biomarker
- Need to identify a biomarker
  - Native chemical
  - Metabolite or adduct
  - Enzyme induction
  - New protein expression
- Validation of the biomarker



# **Practical Limitations of Biomarkers in Humans**

- **Noninvasive**
- **Readily acceptable to test subjects**
- **Inexpensive**
- **Sensitive**



# Potential Biological Samples For Fetal Exposure

- Maternal specimens
  - Urine
  - Hair
  - Blood
  - Breath
  - Transdermal
- Maternal/fetal/neonatal imaging



# Potential Biological Samples For Fetal Exposure

- Newborn Specimens
  - Cord blood
  - Placenta and/or cord
  - Urine
  - Hair
  - Breath
  - Transdermal
  - Amniotic Fluid
  - Meconium

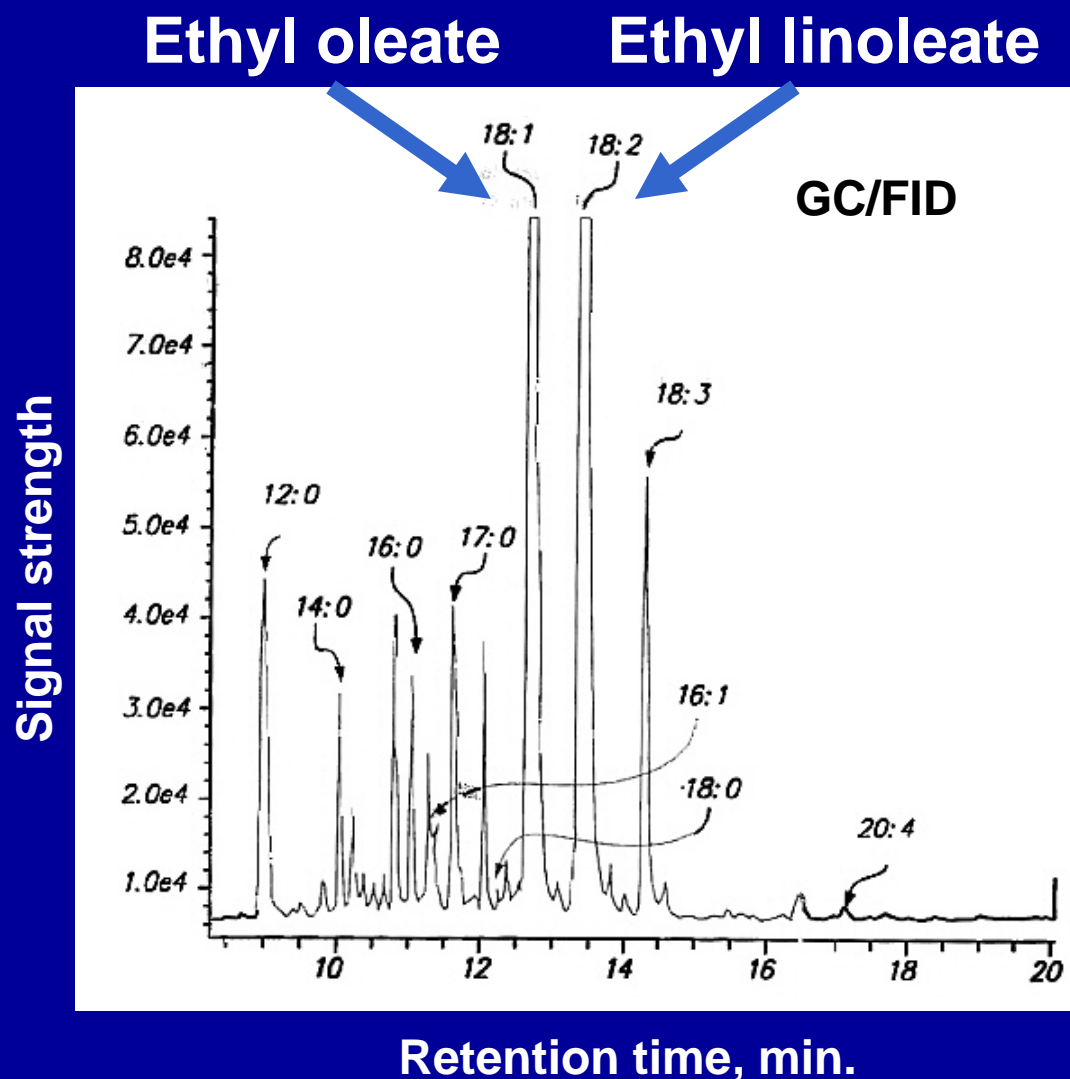


# Fatty Acid Ethyl Esters (FAEE)

- $$\begin{array}{c} \text{R}-\text{C}-\text{OH} \\ \parallel \\ \text{O} \end{array} + \text{HO}-\text{CH}_2-\text{CH}_3 = \begin{array}{c} \text{R}-\text{C}-\text{O}-\text{CH}_2-\text{CH}_3 \\ \parallel \\ \text{O} \end{array}$$
- R = 12:0, 14:0, 16:0, 18:1, 18:2, 18:3, 20:4, 22:6
- Long half life
- Found in fetal tissues
- ? Role in pathogenesis



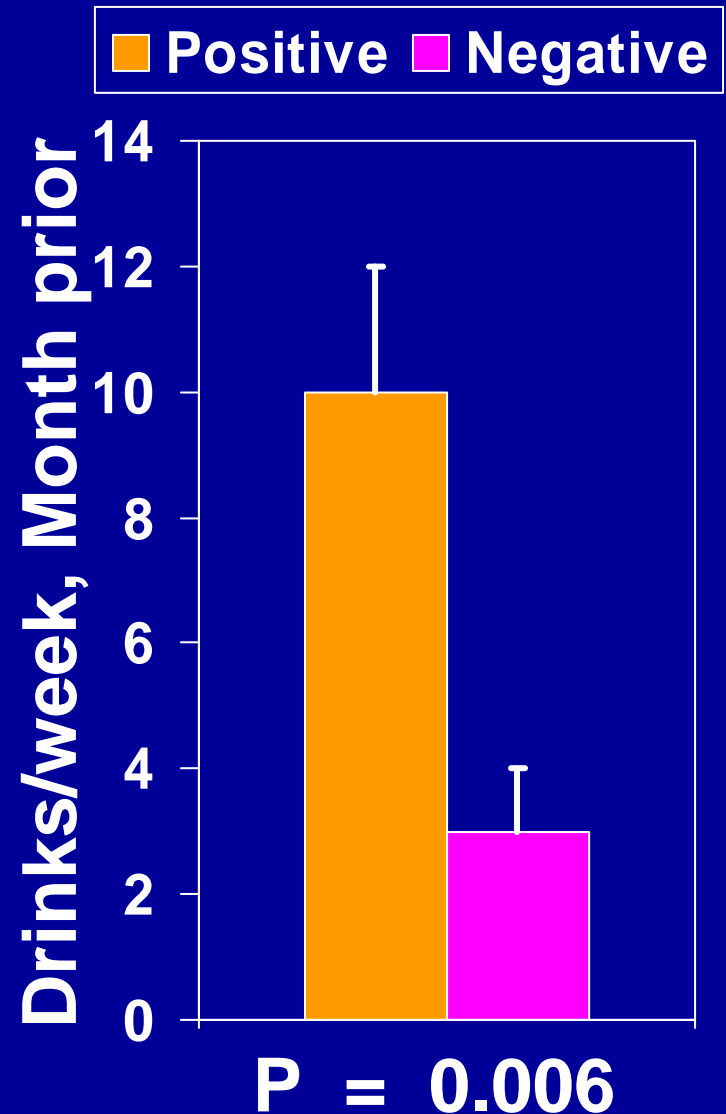
# FAEE: Biomarkers of Prenatal Ethanol Exposure





# Background

- Cleveland population
- 219 meconium samples with maternal history
- Significant difference between reported drinking of mothers who tested positive or negative for ethyl linoleate in meconium



Bearer et al, Alcohol Clin Exp Res, 1999









# Compare FAEE between groups to identify informative FAEE

FAEE	Cleveland Nonabstainers
14:0EE	J, CA
16:0EE	J, CA
16:1EE	None
18:1EE	J, CA
18:2EE	J, CA
18:3EE	J
20:4EE	CA

- J – all  $p < 0.0001$  from Jordan samples
- CA – all  $p < 0.05$  from Cleveland abstainers



# **7 Drinks/drinking day Month prior to pregnancy**

<b>FAEE</b>	<b>AUC</b>	<b>%Sens</b>	<b>%Spec</b>	<b>PPV</b>	<b>NPV</b>
<b>Ethyl oleate</b>	<b>74</b>	<b>92</b>	<b>61</b>	<b>14</b>	<b>99</b>
<b>Ethyl Linoleate</b>	<b>73</b>	<b>85</b>	<b>65</b>	<b>14</b>	<b>98</b>

**Bearer et al, J Pediatr 2005**



# FAEE Associated with Lower PDI

Mean (95% CI) PDI Score at 2 Years				
FAEE	<LOD	< 1 µg/g	≥ 1 µg/g	P value
14:0EE	103 (100-106)	102 (99-105)	99 (94-104)	0.45
16:0EE	102 (94-109)	103 (101-105)	98 (94-102)	0.11
18:1EE	110 (100 – 119)	103 (101-106)	99 (96-102)	0.03
18:2EE	106 (100-111)	103 (101-106)	99 (96-102)	0.02
18:3EE	103 (99-108)	104 (101-106)	98 (95-102)	0.04
20:4EE	103 (100-106)	103 (100-106)	98 (94-101)	0.04



# Association of FAEE with MDI and PDI after controlling for other variables

FAEE	MDI – P value	PDI - P value
Ethyl myristate	0.05	0.02
Ethyl oleate	0.01	<0.01
Ethyl linoleate	<0.01	<0.01
Ethyl linolenate	<0.01	<0.01
Ethyl arachidonate	0.02	0.03



# OUT OF AFRICA



- Capetown Province has the highest known incidence of FAS in the world
- 5 - 8% of all liveborns in one area of South Africa are affected



# **SOUTH AFRICAN STUDY**

- **Prospective detailed alcohol use questionnaires in a population where denial is not expected to be as pronounced**
- **Degree of drinking much larger than in Cleveland sample**
- **Meconium analysis done by GC/MS/MS at CDC (more sensitive/specific)**





# Correlations of Self-Reported Alcohol Consumption with FAEE

	N	AADY	AADD	DDY/WK
Adjusted for wet weight				
Ethyl palmitate	26	0.25	0.34†	0.29
Ethyl oleate	25	0.34†	0.48*	0.32
Ethyl linoleate	27	0.26	0.27	0.29
Adjusted for dry weight				
Ethyl palmitate	26	0.20	0.35†	0.24
Ethyl oleate	25	0.29	0.51**	0.24
Ethyl linoleate	27	0.21	0.27	0.24

† $p < 0.10$ , \* $p < 0.05$ , \*\* $p < 0.01$ .



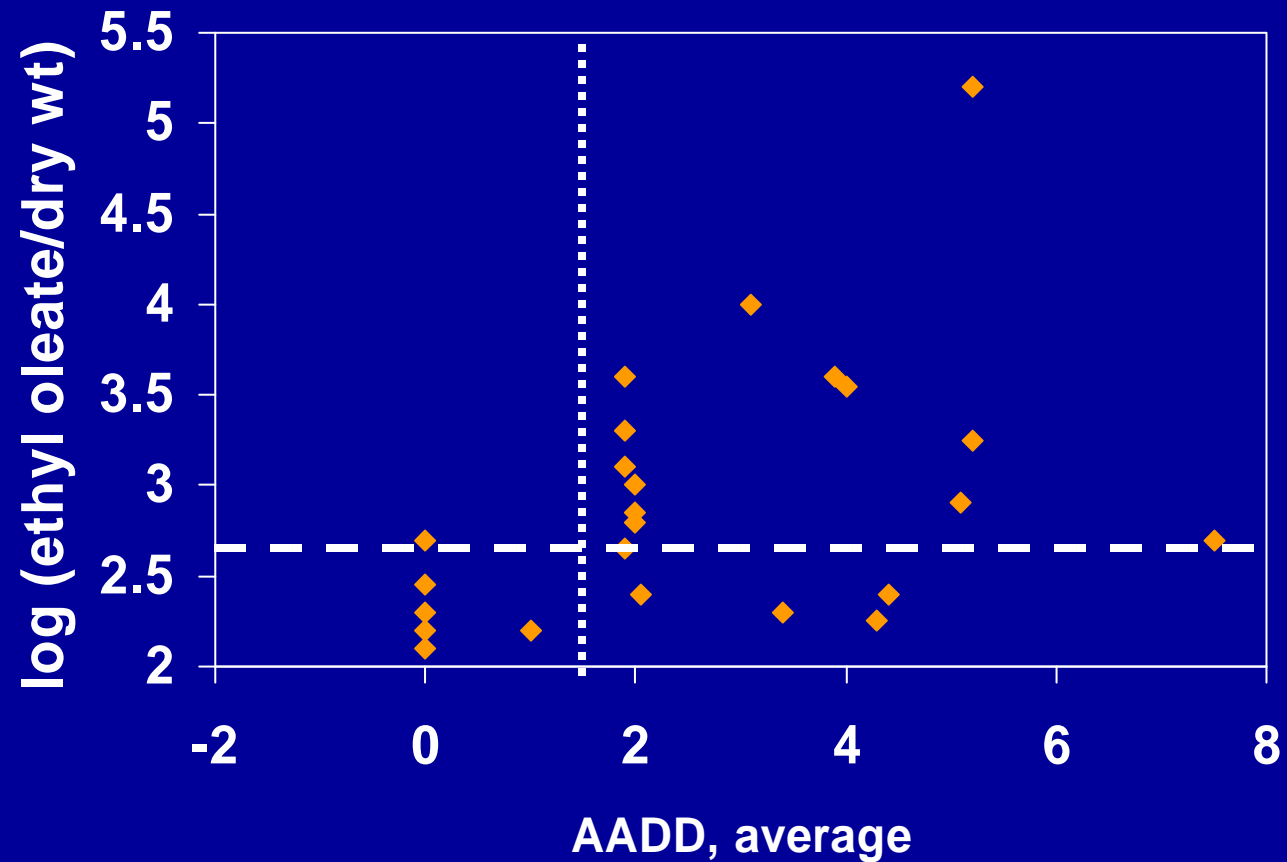
# Correlations Of Self-reported AADD By Trimester With Concentrations Of Ethyl Oleate In Meconium

	N	Pre	1st	2nd	3rd	Ave.
Ethyl oleate						
Wet weight	25	.29	.38†	.52**	.42*	.48*
Dry weight	25	.32	.42*	.55**	.40*	.51**

† $p < 0.10$ , \* $p < 0.05$ , \*\* $p < 0.01$ .



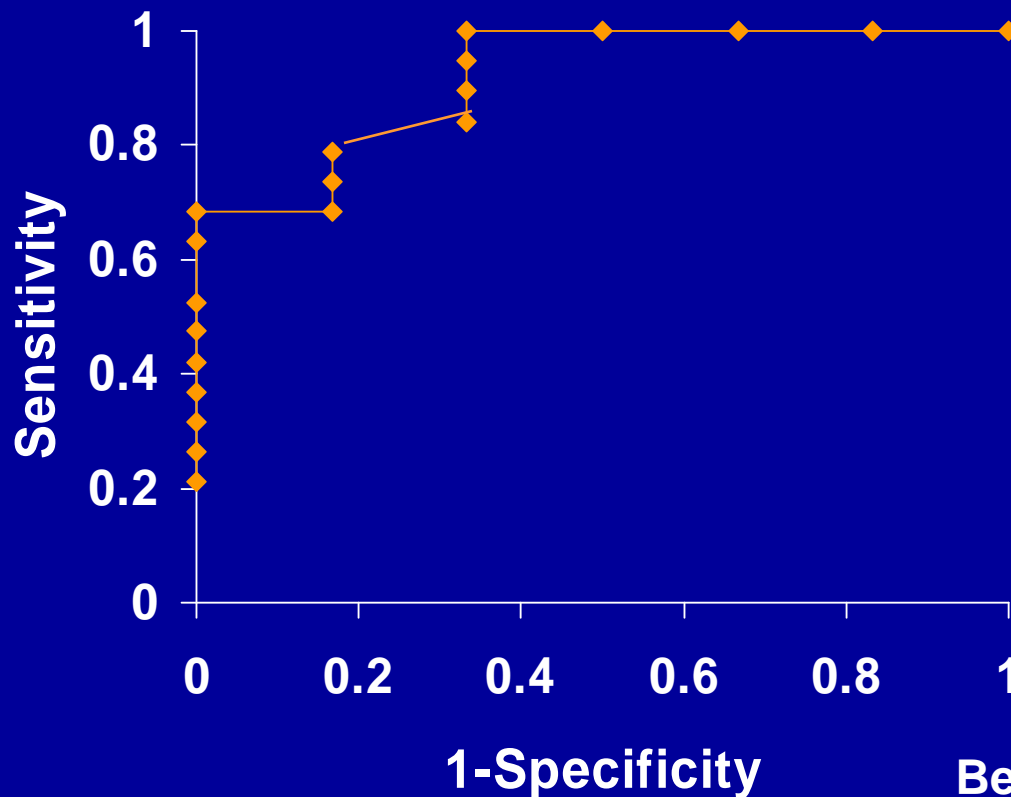
# SCATTERGRAM





# ROC Curve – 3 drinks/episode

	AUC	SE	P	95% CI of AUC
Ethyl Oleate, dry	0.921	0.0602	<0.0001	0.803 to 1.000



Bearer et al, J Pediatr 2003



# Ethyl Oleate - South Africa

## 3 drinks/episode

	AUC %	Sens %	Spec %	PPV %	NPV %	FAEE CO ng/g
Ave.	92	84	83	94	63	32



## Relation of Meconium Ethyl Oleate Concentration (ng/g) to FASD Diagnosis

	<u>M</u>	Standard deviation
FAS/Partial FAS	1862.5 <sup>a</sup>	5545.6
Heavy exposed, nondysmorphic	296.0 <sup>b</sup>	527.0
Non-exposed	11.7 <sup>a,b</sup>	17.1

$F(2, 31)=7.08, p<.01.$

<sup>a</sup>FAS/PFAS group > Non-exposed group,  $p = .001.$

<sup>b</sup>Heavy exposed, nondysmorphic group > Non-exposed group,  
 $p = .004.$



## Relation of Maternal Reported Pregnancy Drinking and Ethyl Oleate to Infant Cognitive Outcomes

	Average Drinks/ AA/day <sup>a</sup>	Drinks/ occasion	Ethyl oleate
FTII novelty preference <sup>b</sup>	-.17* (102)	-.02 (102)	-.48** (26)
A-not-B	-.21* (103)	-.16 <sup>†</sup> (103)	-.27 (28)
Symbolic Play			
Spontaneous	-.18* (114)	-.18* (114)	-.26 (29)
Elicited	-.34*** (117)	-.27** (117)	-.29 (30)

<sup>a</sup>Log transformed. <sup>b</sup>Average for 6.5- and 12-month assessments.

<sup>†</sup> $p < .10$ ; \* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$ .



# FAEE in Sheep Meconium

	Control (ng/g)	Ethanol (ng/g)	P Value
Ethyl oleate	0.28 (0 - 0.63)	1.2 (0.11 - 4.23)	0.001
Ethyl linoleate	0.86 (0.25 - 1.93)	1.23 (0.42 – 1.82)	0.05
Ethyl palmitate	0.34 (0.08 - 0.96)	0.69 (0.07 – 1.98)	0.02

Littner, in press, Pediatr Res



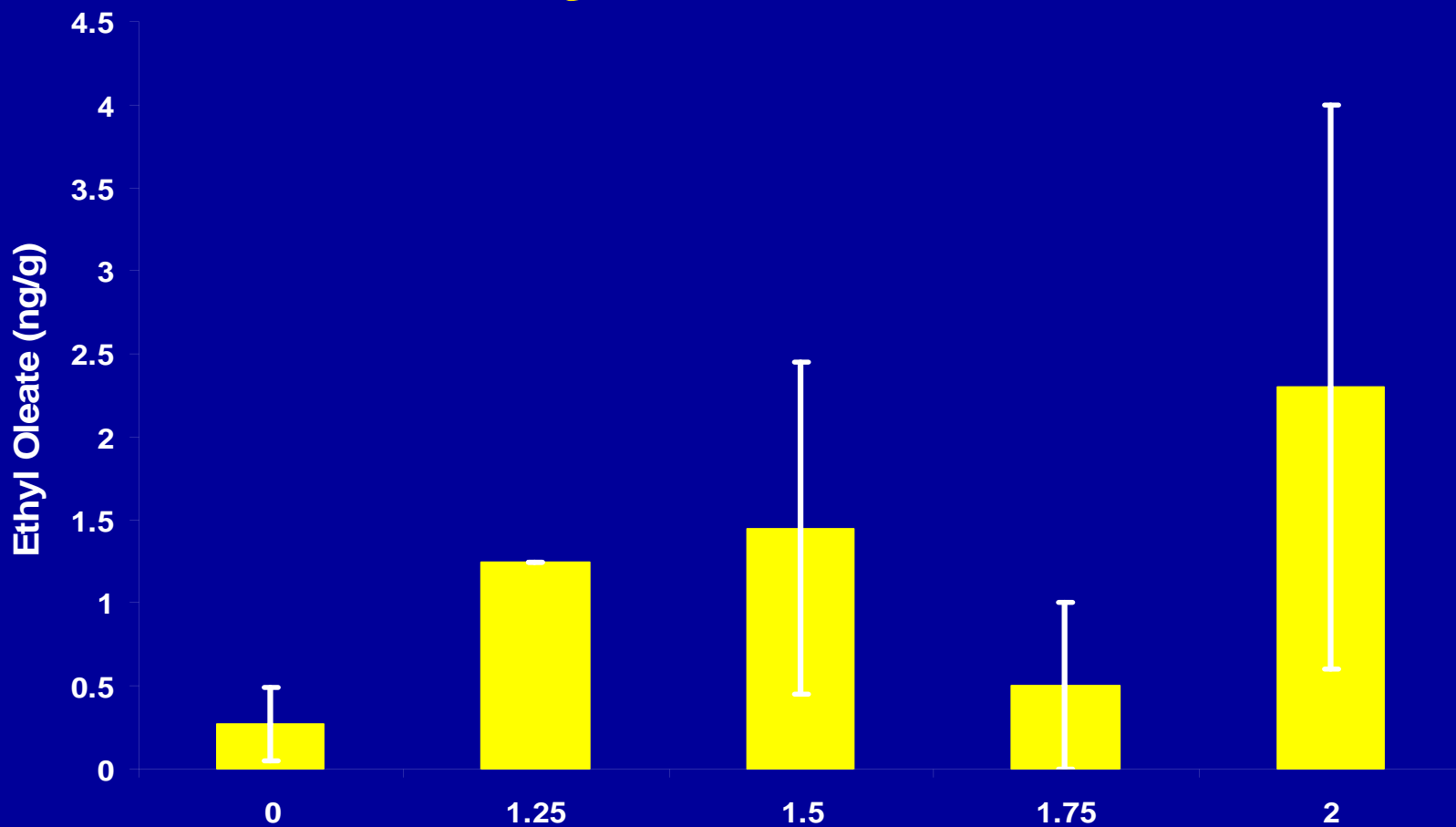


# Sheep Meconium

FAEE	AUC (%)	Cut-off*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Ethyl Myristate 14:0	54	116	56	57	45	67
Ethyl Palmitate 16:0	72	98	67	86	75	80
Ethyl Palmitoleate 16:1	51	339	56	64	50	69
Ethyl Oleate 18:1	94	131	89	100	100	93
Ethyl Linoleate 18:2	75	247	78	79	70	85
Ethyl Linolenate 18:3	71	0	67	79	67	79
Ethyl Arachidonate 20:4	78	0	56	100	100	78
Sum of 3 FAEEs (18:1+18:2+20:4)	88	376	93	89	93	89



# Ethyl Oleate in Sheep Meconium by GC-FID



Ethanol dose, g/kg  
Third trimester, 3 consecutive days/week

Littner, in press, Pediatr Res



# Other Pollutants in Meconium

- Cincinnati's Children's
- 450 mother-infant pairs
- Prospective
- Collect maternal samples/infant samples including meconium
- Collect maternal questionnaire and environmental samples
- 2 year follow up



# Total PCB's

	Maternal Blood Samples during Pregnancy			Infant Samples	
Timing of Samples	16 week	26 week	Birth	Cord Blood	Meconium
Geometric Mean (ng/g)	50.3	42.8	44.0	21.1	46.2
Range (ng/g)	8.6 to 143	6.9 to 190	3.8 to 166	.89 to 121	1.1 to 563
No. (%) Below Detection	None	None	None	None	None



# Total p'p-DDE

	Maternal Blood Samples during Pregnancy			Infant Samples	
Timing of Samples	16 week	26 week	Birth	Cord Blood	Meconium
Geometric Mean (ng/g)	75.6	53.9	59.5	52.5	190.3
Range (ng/g)	29 to 1081	27 to 160	0.93 to 1274	1.82 to 1090	22.9 to 1333
No. (%) Below Detection	None	None	(4%)	9%	None



# Total DDT

	Maternal Blood Samples during Pregnancy			Infant Samples	
Timing of Samples	16 week	26 week	Birth	Cord Blood	Meconium
Geometric Mean (ng/g)	4.1	2.5	3.4	3.5	11.4
Range (ng/g)	0.84 to 82.6	0.78 to 11.7	0.69 to 90.6	2.1 to 55.6	1.6 to 66.8
No. (%) Below Detection	20%	9.3%	18.3%	52.2%	11.9%



# Cost/Benefit Ratios for Universal Meconium Screening

Treatment Type	Total Cost of Screening and Treatment Type for All Test Positives	Benefit Estimate for Prevention Future FAS Births and Maternal Productivity	Benefit Estimate for Early Diagnosis	Total Benefit Estimate (Prevention Future FAS Births, Maternal Productivity and Early Diagnosis)	Total Benefit to Cost Ratio (\$)
Brief Intervention	\$.19 billion	\$9.57 billion	\$.44 billion	\$10.0 billion	52:1
Pharmacotherapy with Medical Management	\$1.37 billion	\$8.07 billion	\$.44 billion	\$8.51 billion	6:1
Residential Facility Treatment	\$4.63 billion	\$9.30 billion	\$.44 billion	\$9.74 billion	2:1



# Future Directions

- Continue analysis of the Cleveland cohort
- Extend the South African study
- Develop a new cohort in Cleveland
- Determine baseline levels of FAEE in nondrinking populations (Muslims, Mormons, Orthodox Jews)
- Continue to develop a sheep animal model





# ACKNOWLEDGEMENTS

- Case
  - L.T. Singer
  - S. Minnes
  - H.L. Kirchner
  - M.A. O'Riordan
  - L.M. Santiago
  - Y. Littner
  - J. Peterson
- Wayne State U.
  - S.W. Jacobson
  - J.L. Jacobson
  - J. Croxford
- CDC
  - Dana Barr
- CHMCC
  - B. Lanphear
- U. of Cape Town
  - C.D. Molteno
  - A. Hay
  - A.S. Marais
- University of Witwatersrand:
  - D.L. Viljoen
- Stanford University
  - H.E. Hoyme
- SUNY Buffalo
  - L.K. Robinson
- Texas A&M University
  - T. Cudd
- Harvard University
  - R.C. Carter

Funded by The Arc, NIAAA, NIEHS, CDC/AAMC